WORLD INTELLECTUAL PROPERTY ORGANIZATION
ORGANISATION MONDIALE DE LA PROPRIÉTÉ INTELLECTUELLE
34, chemin des Colombettes, Case postale 18, CH-1211 Genève 20 (Suisse)
Téléphone: (41 22) 338 91 11 - e-mail: wipo.mail @ wipo.int. - Fac-similé: (41 22) 733 54 28

PATENT COOPERATION TREATY (PCT)
TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

CERTIFIED COPY OF THE INTERNATIONAL APPLICATION AS FILED AND OF ANY CORRECTIONS THERETO

COPIE CERTIFIÉE CONFORME DE LA DEMANDE INTERNATIONALE, TELLE QU'ELLE A ÉTÉ DÉPOSÉE, AINSI QUE DE TOUTES CORRECTIONS Y RELATIVES

International Application No. / Demande internationale n° } PCT/IB 0 3 / 0 1 1 4 6 3

International Filing Date / Date du dépôt international } 18 APRIL 2003
(1 8. 04. 03)

Geneva/Genève, 21 APRIL 2004
(2 1. 04. 04)

International Bureau of the World Intellectual Property Organization (WIPO)

Bureau International de l'Organisation Mondiale de la Propriété Intellectuelle (OMPI)

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

J.-L. Baron
Head, PCT Receiving Office Section
Chef de la section "office récepteur du PCT"

CHA

PCT REQUEST

BR74887/CR

Original (for SUBMISSION) - printed on 18.04.2003 03:30:43 PM

0	For receiving Office use only	
0-1	International Application No.	PCT / IB 0 3 / 0 1 4 6 3
0-2	International Filing Date	18 APRIL 2003 (18.04.03)
0-3	Name of receiving Office and "PCT International Application"	INTERNATIONAL BUREAU OF WIPO PCT International Application
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.04.2003)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	International Bureau of the World Intellectual Property Organization (RO/IB)
0-7	Applicant's or agent's file reference	BR74887/CR
I	Title of invention	METHOD FOR THE TREATMENT OF DISEASES LINKED TO AN ACCUMULATION OF TRIGLYCERIDES AND CHOLESTEROL
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM)
II-5	Address:	101 rue de Tolbiac F-75013 PARIS France
II-6	State of nationality	FR
II-7	State of residence	FR
II-8	Telephone No.	33 1 44 23 63 94
II-9	Facsimile No.	33 1 45 85 07 66
III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	FROMENTY, Bernard
III-1-5	Address:	14 rue de Bellevue F-95160 MONTMORENCY France
III-1-6	State of nationality	FR
III-1-7	State of residence	FR

IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	LE COUPANEC, Pascale
IV-1-2	Address:	NONY & ASSOCIES 3 rue de Penthièvre F-75008 PARIS France
IV-1-3	Telephone No.	33 1 43 12 84 60
IV-1-4	Facsimile No.	33 1 43 12 84 70
IV-1-5	e-mail	nony@nony.fr
V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

PCT REQUEST

BR74887/CR

Original (for SUBMISSION) - printed on 18.04.2003 03:30:43 PM

V-5	Precautionary Designation Statement In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	Exclusion(s) from precautionary designations	NONE	
VI	Priority claim	NONE	
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)	
VIII	Declarations	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	4	-
IX-2	Description	18	-
IX-3	Claims	3	-
IX-4	Abstract	1	EZABST00.TXT
IX-5	Drawings	7	-
IX-7	TOTAL	33	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-17	PCT-EASY diskette	-	Diskette
IX-19	Figure of the drawings which should accompany the abstract		
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative		
X-1-1	Name (LAST, First)	LE COUPANEC, Pascale	

PCT REQUEST

BR74887/CR

Original (for SUBMISSION) - printed on 18.04.2003 03:30:43 PM

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	18 APRIL 2003 (18.04.03)
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

Method for the treatment of diseases linked to an accumulation of triglycerides and cholesterol

The invention relates to a method for the treatment and/or the prevention of diseases linked to an accumulation of triglycerides and cholesterol in tissues and blood of a human or non human animal.

Further, the invention relates to a method for the reduction of body fat mass in a human or non human animal having obese conditions or having a risk to manifest obese conditions.

Hyperlipidemia and obesity afflict an increasing proportion of the population in western societies and may eventually lead to the clinical manifestations of coronary heart diseases, hepatic steatosis (i.e. fatty liver) and type 2 diabetes (i.e. non-insulin dependent diabetes mellitus; NIDDM).

Regarding obesity, it is a chronic disease that is associated with decreased life span and numerous medical problems. In particular, obesity increases the risk of insulin resistance, type 2 diabetes, hepatic steatosis, hyperlipidemia (including elevated levels of plasma triglycerides, cholesterol and free fatty acids), cholelithiasis, hypertension and other cardiovascular diseases. Considering the high prevalence of obesity in population of western societies, any therapeutic drugs potentially useful in reducing weight of body fat could have a profound beneficial effect on their health.

Accordingly, it is one object of the instant invention to provide a therapy for obesity that efficiently reduce or inhibit the gain of body fat mass induced by diets enriched in lipids and carbohydrates.

It is yet another object of the instant invention to prevent obesity, and once treatment has begun, to prevent progression or arrest the onset of diseases that are the consequences of, or secondary to, obesity, such as insulin resistance, type 2 diabetes, hyperlipidemia, fatty liver and cardiovascular diseases.

It is known that excess of fat in the body, including triglycerides and cholesterol in blood, linked or not to obesity condition, is a risk factor for cardiovascular diseases such as angina pectoris, myocardial infarction and hypertension. Hypertension can have a variety of uncomfortable and dangerous side effects and it is seen as a major risk factor in relation to coronary heart diseases. Specific ailments attributable to hypertension

include heart failure, myocardial infarction, rupture or thrombus of the blood vessels in the brain, and kidney damages.

Accordingly, it is still another object of the invention to provide also a treatment for lowering the concentration of body fat, in particular lipids in the blood and/or as a preventive measure in people at risk due to high blood levels of cholesterol and triglycerides.

It is also an object of the present invention to provide a treatment that is useful in lowering the concentration of triglycerides in the liver.

Accumulation of fat in the liver (i.e. hepatic steatosis, also called fatty liver) can be induced by various mechanisms such as excessive mobilization of fatty acids from adipose tissue, decreased hepatic fatty acid oxidation, increased fatty acid and triglyceride synthesis and decreased egress of lipoprotein from the liver (Fromenty B., Pessayre D., 1995 Pharmacol. Ther. 67:101-154). It is noteworthy that different mechanisms can coexist in a same individual. Obesity, insulin resistance, type 2 diabetes and dyslipidemias (including hyperlipidemia) can induce macrovacuolar steatosis which is mainly an accumulation of triglycerides in the hepatocytes. In the long term (i.e. over several years) steatosis can evolve toward steatohepatitis and cirrhosis. Steatohepatitis is characterized by a combination of steatosis (both macrovacuolar and microvesicular), necrosis (or apoptosis), inflammation and fibrosis. Steatohepatitis is a potentially severe liver disease that can lead to cirrhosis, liver failure, hepatocellular carcinoma, and death of the patient.

It has now been found that beta-aminoisobutyric acid (β-aminoisobutyric acid, also called "BAIBA"), presents beneficial effects on lipid homeostasis in obese (ob/ob) and lean (Swiss) mice.

In particular, in feeding experiments in lean mice treated with β-aminoisobutyric acid, the results show that β-aminoisobutyric acid reduces the gain of body fat mass in Swiss mice fed with a standard diet or a western (hypercaloric) diet. In these experiments, β-aminoisobutyric acid significantly decreases the gain of body fat mass by 55 % in mice fed with a standard diet and by 20 % in mice fed with a hypercaloric diet. This makes β-aminoisobutyric acid a potent active agent for the treatment and /or the prevention of obesity.

Further, it has been shown that β-aminoisobutyric acid is efficient for decreasing liver triglycerides and reducing hypertriglyceridemia and hypercholesterolemia.

With this respect, it has been first observed that β-aminoisobutyric acid increases mitochondrial beta-oxidation of fatty acids in liver (results not submitted). In particular, it has been shown that β-aminoisobutyric acid decreases liver and plasma triglycerides in mice fed with a hypercaloric diet and decreases liver triglycerides and plasma phospholipids and cholesterol in genetically obese ob/ob mice fed with a standard diet.

All these results are more precisely disclosed in the following experimental part.

Thus, β-aminoisobutyric acid appears to be particularly useful for the treatment of hyperlipidemic conditions and may be used as a preventive measure in people having risk due to high blood levels of cholesterol and triglycerides and/or suffering from any type of disease linked to the accumulation of triglycerides and cholesterol in tissues and blood.

Accordingly, the present invention relates to a method for the treatment and/or the prevention of diseases linked to the accumulation of triglycerides and cholesterol in tissues and blood comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

The disease may be hyperlipidemia (i.e. hypertriglyceridemia and/or hypercholesterolemia), hepatic steatosis, steatohepatitis and related liver diseases, insulin resistance, type 2 diabetes, syndrome X (i.e. metabolic syndrome), hypertension, angina pectoris and myocardial infarction.

The term "metabolic syndrome" is inter alia characterized by hyperglycemia, central obesity (i.e. accumulation of visceral fat), hepatic steatosis, dyslipidemia and/or hypertension.

β-Aminoisobutyric acid, is a natural β-amino acid generated during thymine metabolism and is also metabolized *in vivo* mainly by the liver and gastrointestinal tissues in metabolites like methylmalonic acid semialdehyde, propionyl-coenzyme A, methylmalonyl-coenzyme A, and succinyl-coenzyme A (Griffith O.W., 1986, Annu. Rev. Biochem. 55 : 855-878).

β-Aminoisobutyric acid may be used directly as active agent or may be generated in vivo after administration of a prodrug thereof like for example thymine or one of its intermediate metabolites.

The instant invention covers also the use of derivatives of β-aminoisobutyric acid.

The term "derivatives" include salts, esters or amides of β-aminoisobutyric acid.

The terminal carboxylic group of β-aminoisobutyric acid may be in particular under the form of an ester, for example lower alkyl ester, (in particular in C_1-C_{10}) or of an amide.

More generally, its salts include not only the addition salts with carboxylic organic acids, like the acetate for example, but also other addition salts such as for example the trifluoroacetate, as well as the addition salts with inorganic acids such as the sulphate, hydrochloride and the like. The derivatives also include the salts resulting from the salivation of the carboxyl group and in particular the salts of alkali metals or alkaline earth metals such as the salts of sodium or of calcium.

In particular, the active agent is β-aminoisobutyric acid. It may be of L (i.e. S) or D (i.e. R) configuration or a mixture of L and D configurations.

A further aspect of the invention relates to a method of treatment for the reduction or inhibition of the gain of body fat comprising at least the step of administering to human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

Still a further aspect of the invention relates to a method of treatment for lowering the blood levels of cholesterol and/or triglycerides comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

Another aspect of the invention relates to a method of treatment for lowering liver level of triglycerides comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

A further aspect of the invention relates to a method for the treatment or prevention of an obese condition, said method comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

The term "obesity" normally refers to a condition whereby an animal has an unusually elevated body fat mass resulting in an abnormally high Body Mass Index (BMI). As an example, adult humans are considered as obese if their BMI is above 30 kg/m^2.

In relation to obesity, the term "treatment" refers to a reduction of the severity of the disease, e.g. by reducing the body fat mass. The body fat mass refers to the total amount of lipids in an organism. These lipids include triglycerides, free fatty acids, cholesterol and cholesterol esters and phospholipids.

In relation to obesity, the term "prevention" refers to preventing obesity from occurring, i.e. β-aminoisobutyric acid is administered prior to the onset of the obese condition. This means that the compounds of the present invention can be used as prophylactic agents to impede an increase in body fat.

The term "animals" includes mammals such as humans and farm (agricultural) animals, especially the animals of economic importance such as gallinaceous birds, bovine, ovine, caprine and porcine mammals, especially those that produce products suitable for the human consumption, such as meat, eggs and milk. Further, the term is intended to include fish and shellfish, such as salmon, cod, Tilapia, clams and oysters. The term also includes domestic animals such as dogs and cats.

In accordance with the methods indicated above, preferred embodiments are as follows: said animal is a human, an agricultural animal and/or a domestic or pet animal.

The treatment involves administering to an animal in need of such a treatment, a therapeutically effective amount of β-aminoisobutyric acid in the blood of the animal for the duration of the period of its administration.

A further aspect of the invention relates to the use of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof, for the preparation of a pharmaceutical composition for the treatment and/or prevention of diseases linked to an accumulation of triglycerides and/or cholesterol in tissues and blood.

The pharmaceutical composition according to the present invention is more particularly directed to the treatment and/or the prevention of hyperlipidemia (i.e. hypertriglyceridemia and/or hypercholesterolemia), hepatic steatosis, steatohepatitis, diabetes, metabolic syndrome, hypertension, angina pectoris and myocardial infarction.

A further aspect of the invention relates to a pharmaceutical composition, in particular useful for the prevention and/or treatment of diseases linked to an accumulation

of cholesterol and triglycerides in tissues and blood, comprising at least an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

The pharmaceutical composition according to the invention is more particularly useful for the treatment or prevention of hypertension, fatty liver, metabolic syndrome and an obese condition.

Preferably, the pharmaceutical composition comprises in admixture with β-aminoisobutyric acid a pharmaceutically acceptable carrier or excipient.

The invention also relates to a nutritional composition comprising an amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof effective to reduce, or to prevent, an increase in the total body fat mass in human or non-human animal, and a method for producing reduction of the fat mass in a human or non-human animal in need thereof, comprising administering thereto an effective amount of said nutritional composition.

The nutritional composition may be any food composition. In particular, it may be a drink or a powder that can be reconstituted to produce such a drink. It may include other nutritional components like vitamins, stabilisers, antioxidants, emulsifiers, flavowing agents.

Preferred embodiments relate to a condition wherein the animal has developed an obese condition or is low energy adapted.

The term "low energy adapted" refers to a condition whereby an animal has a low energy consumption, i.e. less than normal.

As a pharmaceutical medicament, the compounds of the present invention may be administered directly to the animal by any suitable technique, including parenterally, intranasally, orally, or by absorption through the skin. They can be administered locally or systemically. The specific route of administration of each agent will depend, e.g., on the medical history of the animal.

Examples of parenteral administration include subcutaneous, intramuscular, intravenous, intraarterial, and intraperitoneal administration.

The term "effective amount" means the minimal amount necessary to observe the expected effect i.e. a lowering effect on the concentration of triglycerides and/or cholesterol in tissues and blood.

In particular, it might be in the range of about 5 mg/kg/day to 1000 mg/kg/day of patient body weight, in particular about 50 mg/kg/day to 500 mg/kg/day.

Generally, the formulations are prepared by contacting the compounds of the present invention each uniformly and intimately with liquid carriers or finely divided solid carriers or both.

In addition, the compounds of the present invention may be appropriately administered in combination with other treatments for combating or preventing the diseases considered according to the invention and/or the obesity.

The invention will be more fully understand by reference to the following examples. They should not, however, be constituted by limiting the scope of the invention.

Legends of the figures

Figure 1: it shows effects of β-aminoisobutyric acid (BAIBA) on body fat mass in Swiss mice fed with a standard diet, with or without BAIBA in the drinking water, and fasted for 48 hours before DEXA measurements (i.e. 2 and 6 weeks after the initiation of the treatment). Results are expressed as the percentage of the control values. Asterisk (*) indicates a significant difference ($p<0.01$) between the groups.

Figure 2: it shows effects of β-aminoisobutyric acid (BAIBA) on the gain of body fat mass (as assessed by DEXA) in Swiss mice fed with a standard diet with or without BAIBA in the drinking water (8 mice in each group). Variations observed for the whole period of the investigation (T0-Tsix weeks) are also shown. Results are expressed in gram. Asterisk (*) indicates a significant difference ($p<0.01$) between the groups.

Figure 3: it shows effects of β-aminoisobutyric acid (BAIBA) on the gain of body fat mass (as assessed by DEXA) in Swiss mice fed with a western diet (WD) with or without BAIBA in the drinking water (8 mice in each group). For comparison, the experiment included a group of mice (n=8) fed with a standard diet (SD). Variations observed for the entire period of the investigation (T0-Tsix weeks) are also shown. Results are expressed in gram. Asterisk (*) indicates a significant difference ($p<0.01$) between Control-WD and BAIBA-WD mice.

Figure 4: it shows effects of β-aminoisobutyric acid (BAIBA) on liver lipids in Swiss mice fed with a western diet (WD) with or without BAIBA in the drinking water. For comparison, the experiment included a group of mice fed with a standard diet (SD).

Mice in this experiment are those studied in Figure 3 but after the last DEXA measurement (i.e. after 6 weeks) animals were fasted for 48 hours and killed for hepatic lipid determination. Total lipids (mg/whole liver) and triglycerides (mg/whole liver) were determined in 7, 8 and 8 mice, respectively in Controls-SD, Controls-WD and BAIBA-WD mice. Asterisk (*) indicates a significant difference ($p<0.05$) between Control-WD and BAIBA-WD mice.

Figure 5: it shows effects of β-aminoisobutyric acid (BAIBA) on liver triglycerides in obese (ob/ob) mice were fed with a standard diet, with (10 mice) or without (7 mice) BAIBA in the drinking water. After 6 weeks of investigation, animals were fasted for 48 hours and killed for hepatic lipid determination. The results are expressed as mg/whole liver and mg/gram of lipids. Asterisk (*) indicates a significant difference ($p<0.01$) between control mice and mice receiving BAIBA.

Figure 6: it shows effects of β-aminoisobutyric acid (BAIBA) on plasma lipids in Swiss mice fed with a western diet (WD) with or without BAIBA in the drinking water. For comparison, the experiment included a group of mice fed with a standard diet (SD). Mice in this experiment are those studied in Figures 3 and 4 (i.e. after 6 weeks of treatment and the last DEXA measurement blood was collected in fasted mice before killing for liver lipid determination). Plasma triglycerides (TG), phospholipids (Ph.L), total cholesterol (Chol) and non-esterified fatty acids (NEFA) were determined in 8 mice for each group (Controls-SD, Controls-WD and BAIBA-WD).

Figure 7: it shows effects of β-aminoisobutyric acid (BAIBA) on plasma lipids in genetically obese (ob/ob) mice fed with a standard diet, with (10 mice) or without (7 mice) BAIBA in the drinking water. After 6 weeks, mice were fasted for 48 hours and blood was collected for the determination of plasma triglycerides (TG), phospholipids (Ph.L) and total cholesterol (Chol). Mice in this experiment are those studied in Figure 5. Asterisk (*) indicates a significant difference ($p<0.05$) between control ob/ob mice and ob/ob mice receiving BAIBA.

Materials and methods

Six to 8 weeks old male (Crl:CD-1(ICR)BR) Swiss mice (i.e. lean mice), weighing 28 and 32 grams were purchased from Dépré (Saint Doulchard, France). Ten to 12 weeks old ob/ob male (C57BL/6-ob) mice (i.e. obese mice), weighing 40 to 44 grams

were purchased from Janvier (Le-Genest-St-Isle, France). In genetically obese ob/ob mice, a mutation on the gene of leptin prevents the normal production of this hormone, thus increasing appetite and decreasing energy consumption (Friedman J.M., Halaas J.L., 1998, Nature 395 : 763-770). Accordingly, ob/ob mice exhibit severe (i.e. "morbid") obesity, massive steatosis, insulin resistance and diabetes (Friedman and Halaas, 1998 see previously; Koteish A., Diehl A.M., 2001, Semin. Liver Dis. 21 : 89-104). The animals were acclimatized for one to two weeks before the start of experiments.

In our investigations, two kinds of diet were used: 1- a standard diet (A04 diet from UAR) containing 3% of lipids and bringing 2900 kcal per kg; 2- a western diet (from UAR too) containing 16% of lipids and bringing 4300 kcal per kg.

D,L-β-Aminoisobutyric acid (BAIBA) was purchased from Sigma-Aldrich. BAIBA has been administered in drinking water at the dose of 100 mg/kg/day for 6 weeks.

The *in vivo* determination of body fat mass in anesthetized mice has been performed by "DEXA" (Dual Energy X-ray Absorptiometry) (Pietriobelli A., Formica C., Wang Z., Heymsfield S.B., 1996, Am. J. Physiol. 271 (Endocrinol. Metab. 34) : E941-E951)). Hence, in our study, the body fat mass is the total amount of lipids (in grams) per animal (excluding its head) that is quantified by DEXA. It is noteworthy that DEXA also gives for each investigated animal its lean mass that is the total amount of water and proteins (in grams) in the body (excluding the head). Therefore, for a given animal, DEXA allows the determination of the percentage of body fat which is its body fat mass divided by the sum of its body lean and fat masses (fat mass/(lean mass + fat mass)). The "DEXA" apparatus was a Piximus® from Lunar Corporation (Madison, WI). Mice were anesthetized thanks to a mixture of xylazine and ketamine.

In this study, two types of procedures were used, according to the nutritional state of animals at the moment of "DEXA" measurements:

Procedure 1: the animals were not fasted before DEXA investigations. The first DEXA measurement was performed one day before the beginning of the investigation (T0). Afterwards, DEXA measurements have been respectively performed two weeks (T2) and six weeks (T6) after the beginning of the treatment. With this procedure, it has been possible to determine in each group of animals the evolution of several parameters (e.g. fat mass, body weight) between respectively T0 and T2, and T2 and T6. Comparisons of these evolutions were thus performed for the T0-T2, T2-T6 and T0-T6 periods.

Procedure 2: the animals were fasted for 48 hours before DEXA measurements which have been performed 2 weeks (T2) and 6 weeks (T6) after starting the treatment. In this procedure, DEXA measurement before the beginning of treatment has not been performed. Comparisons of the different parameters (e.g. fat mass, body weight) between both groups were performed at T2 and at T6.

Total lipids and triglycerides in the liver of animals were assessed according to a procedure partially reported by (Lettéron P., Fromenty B., Terris B., Degott C., Pessayre D., 1996, J. Hepatol. 24 : 200-208). Briefly, after killing of the animals, livers were removed and homogenized in sterile water. Hepatic lipids were thus extracted by a mixture of chloroform and methanol (2/1; v/v). After removal of the aqueous phase, the organic phase (chloroform containing the lipids) was evaporated and the amount of lipids was determined by gravimetry. Lipids were subsequently resuspended in isopropanol (final concentration, ca. 10 mg/mL). After removal of the phopholipids by using aluminum hydroxide hydrate, triglycerides were determined colorimetrically by using periodate and the Nash's reagent (containing acetylacetone, ammonium acetate and isopropanol). The reaction generates diacetyldihydrolutidine which is assayed by spectrophotometry ($\lambda = 410$ nm).

Plasma lipids (triglycerides, total cholesterol, phospholipids) were measured on an automatic analyser (Hitachi 717®). The commercial kits used to assess triglycerides, total cholesterol and phospholipids on this analyser were all from bioMérieux (references 61238, 61219 and 61491, respectively). Plasma non esterified fatty acids (NEFA) were assessed by using a commercial kit (Wako, reference 994-75409). Hepatic and plasma lipids were performed at the end of six weeks of treatment in animals that have been fasted for 48 hours.

The results are expressed as mean ± SEM (standard error of the mean). A *t* test of Student was used to look for a statistic difference between values obtained for the control group and the group of mice treated with BAIBA. This difference was considered as statically significant for a value of $p<0.05$. The numbers indicated between the parentheses represent the numbers of animals used in the experiments.

EXAMPLE 1

Beneficial effects of BAIBA on body fat

a) Effect of BAIBA in lean mice after a fasting period

In a first series of experiments, the effects of β-aminoisobutyric acid (BAIBA) on body fat were investigated in Swiss (lean) mice fed with a standard diet (A04 chow) and fasted for 48 hours before DEXA measurements. Mice were 6 to 8 weeks old at the beginning of the experiment. DEXA was performed twice after the initiation of the treatment. In this experiment, BAIBA decreased body fat mass by 31 % and 25%, respectively after 2 and 6 weeks of treatment. The results in figure 1 indicate that:

- after 2 weeks, body fat mass was 2.63 ± 0.23 and 1.80 ± 0.11 grams, respectively in control mice (n=7) and mice receiving BAIBA (n=7);
- after 6 weeks, body fat mass was 3.40 ± 0.27 and 2.56 ± 0.32 grams, respectively in control mice and mice receiving BAIBA.

In this experiment, body weight was not significantly different between control mice and mice receiving BAIBA, after 2 and 6 weeks of treatment (data not shown).

b) – Effect of BAIBA in lean mice not submitted to fast

In a second series of investigations, we sought to determine as to whether β-aminoisobutyric acid (BAIBA) decreases body fat in Swiss mice not submitted to fast before DEXA measurements. Mice were 6 to 8 weeks old at the beginning of the experiment. In this experiment, DEXA was performed one day before the beginning of the treatment (T0) and then two and six weeks after the initiation of the experience (T2 and T6, respectively). This allowed us to determine for each animal the variation of body fat mass for the different periods of the treatment (T0-T2, T2-T6 and T0-T6). In this experiment, we found that BAIBA reduced the gain of body fat mass in Swiss mice and that this effect was more pronounced in the second period (T2-T6) of the treatment. This effect of BAIBA is submitted in figure 2. It shows that:

- through the T0-T2 period, control mice (n=8) gained 0.76 ± 0.06 grams of body fat whereas mice receiving BAIBA (n=8) gained 0.56 ± 0.12 grams.
- through the T2-T6 period, control mice gained 1.10 ± 0.07 grams of body fat whereas mice receiving BAIBA gained 0.28 ± 0.12 grams.
- Overall, for the entire period of the experiment (T0-T6), BAIBA reduced the gain of fat mass by 55%, as control mice and mice receiving BAIBA gained respectively 1.86 ± 0.11 and 0.84 ± 0.18 grams of body fat.

In this experiment, BAIBA slowed the gain of body weight during the 6-week period of treatment. Indeed, through the T0-T6 period, BAIBA significantly ($p<0.01$) reduced the gain of body weight by 12%, as control mice and mice receiving BAIBA gained respectively 10.30 ± 0.34 and 9.02 ± 0.42 grams.

c) – Effect of BAIBA in lean mice fed with a Western diet

In a third series of experiments, we sought to determine as to whether β-aminoisobutyric acid (BAIBA) would be able to decrease body fat mass in Swiss mice fed with a western diet (i.e. hypercaloric diet). For this purpose, young (4 weeks old at T0) Swiss mice were fed with a hypercaloric western diet (WD), with or without BAIBA in the drinking water (8 mice in each group). For comparison, the experiment included a group of mice (n=8) fed with a standard diet (SD). DEXA was performed one day before the beginning of the treatment (T0) and then two, four and six weeks after the initiation of the experience (T2, T4 and T6, respectively). This allowed us to determine for each animal the variation of body parameters (e.g. fat mass, body weight) for the different periods of the treatment (T0-T2, T2-T4, T4-T6 and T0-T6). For these four DEXA determinations (T0 to T6), mice were not fasted before measurements. In this experiment, BAIBA reduced the gain of body fat mass in Swiss mice fed with a western diet and this beneficial effect was observed from the fourth week of the BAIBA administration. This effect is illustrated in figure 3. It shows that:

- during the T4-T6 period control mice fed with the western diet gained 1.06 ± 0.34 grams of body fat whereas mice fed with the same diet and receiving BAIBA lost 0.45 ± 0.30 grams of body fat (significantly different, $p<0.05$),
- through the entire period of the treatment (T0-T6), the gain of body fat mass was reduced by 20% in mice fed with the western diet and receiving BAIBA. Indeed, during the T0-T6 period control mice fed with the western diet (Controls-WD) gained 4.88 ± 0.83 grams of body fat whereas mice fed with the same diet and receiving BAIBA (BAIBA-WD) gained 3.91 ± 0.59 grams of body fat mass. For comparison, mice fed with the standard diet (Controls-SD) gained 3.18 ± 0.34 grams of body fat during the entire period (T0-T6) of the experiment.
- At the end of the treatment (T6), the body fat mass was 4.48 ± 0.33, 6.21 ± 0.85 and 5.25 ± 0.60 grams, respectively for the Controls-SD, Controls-WD and BAIBA-

WD groups of mice (15% decrease between Controls-WD and BAIBA-WD). The percentage of body fat ((fat mass)/(fat+lean mass)) was at this time 13.23 ± 0.86, 17.96 ± 2.04 et 15.76 ± 1.53 %, respectively in the Controls-SD, Controls-WD and BAIBA-WD groups. Altogether, these results indicate that the western diet increased body fat mass in Swiss mice over a 6-week period and that BAIBA was able to curb this abnormal gain of body fat.

In this experiment, BAIBA tended to reduce body weight in mice fed with the western diet. Indeed, after the 6-week period of treatment body weight was 37.9 ± 1.14 and 37.0 ± 0.76 grams, respectively in control mice (Controls-WD) and mice receiving BAIBA (BAIBA-WD).

Finally, we sought to determine on the very same animals as to whether fasting would be able to enhance the beneficial effect of BAIBA on body fat mass. Thus, after the fourth DEXA measurement (i.e. DEXA determination at T6), mice were allowed to recover from the anaesthesia for ca. 2 days and were then submitted to a 48-hour period of fast. A last DEXA measurement was then performed in fasted mice. Our results showed that fasting did not increase the beneficial effect of BAIBA. Indeed, after the fasting period, body fat mass was 3.10 ± 0.30, 5.40 ± 0.75 et 4.79 ± 0.56 grams, respectively in control mice fed the standard diet (Controls-SD), control mice fed with the western diet (Controls-WD), and mice fed with the western diet and receiving BAIBA (BAIBA-WD). Thus, in the animals fed with the western diet for 6 weeks, BAIBA decreased by 15 and 11% the percentage of body fat (determined at T6 or a few days later), respectively in the fed and the fasted state. These results indicate that a reduction of food intake does not provide further benefit to the favourable effect of BAIBA on body fat. At the end of the fasting period, body weight was 32.0 ± 0.6, 34.2 ± 1.1 and 33.6 ± 0.9 grams, respectively in the Controls-SD (n=8), Controls-WD (n=8), and BAIBA-WD (n=8) groups of mice.

Another experiment has been started in Swiss mice (4 weeks old at T0) fed with the western diet with the aim to assess the effect of BAIBA over a longer period of treatment. At the moment, DEXA measurements have been performed before the beginning of the treatment (T0) and 1 month later (T1m). For the T0-T1m period of this new experiment, the gain of body fat was reduced by 12% in mice fed with the western diet and receiving BAIBA. Indeed, control mice fed with the western diet (Controls-WD, n=8) gained 4.89 ± 0.64 grams of body fat whereas mice fed with the same diet and

receiving BAIBA (BAIBA-WD, n=9) gained 4.28 ± 0.47 grams of body fat mass. For comparison, mice fed with the standard (Controls-SD, n=8) diet gained 3.36 ± 0.29 grams of body fat during the same period (T0-T1m) of the experiment. Thus, in this new experiment, although the beneficial effect of BAIBA on body fat was moderate, this effect was observed sooner when compared with the investigation reported above (Figure 3).

d)- Effect of BAIBA in genetically obese ob/ob mice:

In a last series of investigations, we sought to determine as to whether β-aminoisobutyric acid (BAIBA) would be able to decrease body fat mass in genetically obese ob/ob mice. In these investigations 6 weeks and 10-12 weeks old ob/ob mice were fed with a standard diet, and DEXA measurements were performed one day before the beginning of the treatment (T0) and then two and six weeks after the initiation of the experience (T2 and T6, respectively). DEXA was performed on fed mice. Our results showed that BAIBA presented a slight beneficial effect on body fat only in the older animals (10-12 weeks old). Indeed, in these mice BAIBA afforded an 11% reduction of body fat in the first two weeks of treatment, since control (n=6) and treated (n=6) mice respectively gained 5.20 ± 0.63 and 4.65 ± 0.35 grams (not significantly different). This beneficial effect of BAIBA was no longer observed during the T2-T6 period, but for the entire period of the experiment the gain of fat mass was reduced by 2%. Indeed, during the T0-T6 period, control and treated mice respectively gained 7.43 ± 0.51 and 7.30 ± 0.61 grams of body fat. It is noteworthy that despite the limited effect of BAIBA on body fat mass in ob/ob mice at the end of the 6-week period of treatment, further investigations show that BAIBA afforded a proportional stronger reduction of liver triglycerides and plasma cholesterol after 6 weeks of investigation (see below).

EXAMPLE II

Beneficial effects of BAIBA on liver lipids

a) - Effect of BAIBA in lean mice fed with a western diet:

Liver lipids were assessed in mice fed with the western diet (WD) receiving or not β-aminoisobutyric acid (BAIBA) in the drinking water (8 mice in each group). For comparison, the experiment also included a group of mice (n=8) fed with a standard diet (SD). These animals were those used for the determination of body fat by DEXA (see

above, paragraph I-c) but after the last DEXA measurement (i.e. after 6 weeks of treatment), mice were allowed to recover from the anaesthesia for ca. 2 days and were then submitted to a 48-hour period of fast. At the end of this fasting period DEXA was again performed (see above) and then mice were killed for liver lipids determination. The western diet was responsible for an increase in liver lipids as compared to mice fed with the standard diet. In the animals fed with the western diet, BAIBA was found to decrease total lipids and triglycerides, respectively by 24 and 17%. This effect is illustrated in figure 4 which shows that:

- liver lipids were 119 ± 16, 142 ± 13 and 108 ± 7 mg/whole liver, respectively in control mice fed the standard diet (Controls-SD, 7 mice), control mice fed with the western diet (Controls-WD, 8 mice), and mice fed with the western diet and receiving BAIBA (BAIBA-WD, 8 mice);

- hepatic triglycerides were 36 ± 10, 40 ± 8 and 33 ± 6 mg/whole liver, respectively in the Controls-SD, Controls-WD and BAIBA-WD groups. Taken together, these results indicate that although the western diet increased liver lipids and triglycerides only modestly during the 6 weeks of the investigations, BAIBA administration was able to fully prevent lipid accumulation in the liver during this period of time.

b) - Effect of BAIBA in genetically obese ob/ob mice:

Liver triglycerides were assessed in ob/ob mice fed with a standard diet and receiving or not β-aminoisobutyric acid (BAIBA) in the drinking water during 6 weeks. Mice were 10 weeks old at the beginning of the experiment. These animals (7 controls and 10 treated with BAIBA) were different from those used for the determination of body fat by DEXA in the fasted state (see above, paragraph I-d). At the end of the 6 weeks of treatment mice were submitted to a 48-hour period of fast. At the end of this fasting period mice were killed for assessment of liver triglycerides. Obese ob/ob mice present a massive accumulation of triglycerides in the liver, and BAIBA was found to reduce hepatic triglyceride levels. Indeed, liver triglycerides were reduced by 16 and 12%, respectively when the values were expressed as mg/whole liver or as mg/gram of lipids. The results are submitted in figure 5 which shows that BAIBA afforded a significant reduction ($p<0.01$) of liver triglycerides from 709 ± 18 to 625 ± 14 mg/g of lipids. Although the diminution of

hepatic triglycerides was moderate, these results suggest that BAIBA present some beneficial effects in ob/ob mice, a model of morbid obesity and massive hepatic steatosis.

EXAMPLE III

Beneficial effects of BAIBA on plasma lipids

a) - Effect of BAIBA in lean mice fed with a western diet:

Plasma lipids were assessed in mice fed with the western diet (WD) and receiving or not β-aminoisobutyric acid (BAIBA) in the drinking water (8 mice in each group). For comparison, the experiment also included a group of mice (n=8) fed with a standard diet (SD). These animals were those used for the determination of body fat by DEXA and liver lipids (see above, paragraph I-c and II-a, respectively). After the last DEXA measurement (after 6 weeks of treatment), mice were allowed to recover from the anaesthesia for ca. 2 days and were then submitted to a 48-hour period of fast. At the end of this fasting period, a sample of blood was collected (from the retroorbital sinus) before DEXA measurements and plasma triglycerides, phospholipids, total cholesterol and non-esterified fatty acids (NEFA) were subsequently determined. After 6 weeks, the western diet was responsible for an increase of all plasma lipids, but the most affected lipids were triglycerides. The results are shown in figure 6.

Interestingly, BAIBA was found to reduce by 22% plasma triglycerides in mice fed with the western diet, although the difference was not significant between control and treated mice (Figure 6). Indeed, plasma triglycerides were 0.88 ± 0.07, 1.96 ± 0.45 et 1.54 ± 0.13 mmol/L, respectively in control mice fed the standard diet (Controls-SD), control mice fed with the western diet (Controls-WD), and mice fed with the western diet and receiving BAIBA (BAIBA-WD). The western diet was also responsible for an increase in plasma NEFA and BAIBA tended to decrease by 16% NEFA in mice fed with this diet (Figure 6). Indeed, plasma NEFA were 0.81 ± 0.09, 1.05 ± 0.08 and 0.88 ± 0.06 mmol/L, respectively in the Controls-SD, Controls-WD and BAIBA-WD groups. Finally, BAIBA tended to decrease total cholesterol by 4% in mice fed with the western diet (from 6.98 ± 0.64 to 6.71 ± 0.27 mmol/L)(Figure 6). All together, these results suggest that BAIBA is able to reduce plasma triglycerides when these lipids are abnormally increased by a western diet.

b) - Effect of BAIBA in genetically obese ob/ob mice:

Plasma lipids were assessed in ob/ob mice fed with a standard diet and receiving or not β-aminoisobutyric acid (BAIBA) in the drinking water during 6 weeks. These animals (7 controls and 10 treated with BAIBA) were those used for the determination of hepatic lipids (see above, paragraph II-b). At the end of the 6 weeks of treatment, mice were submitted to a 48-hour period of fast. Samples of blood were then collected (from the retroorbital sinus) for the assessment of plasma triglycerides, phospholipids and total cholesterol and mice were subsequently killed for assessment of liver triglycerides (see above). In this experiment, BAIBA decreased by 5, 23 and 19%, respectively plasma triglycerides, phospholipids and total cholesterol. The results are submitted in figure 7. They show that:

- plasma triglycerides were 1.39 ± 0.22 and 1.32 ± 0.14 mmol/L, respectively in control and treated ob/ob mice;
- plasma phospholipids were significantly decreased by BAIBA from 5.27 ± 0.32 to 4.07 ± 0.26 mmol/L ($p<0.01$);
- plasma total cholesterol was significantly decreased by BAIBA from 6.13 ± 0.34 to 4.98 ± 0.34 mmol/L ($p<0.05$).

Altogether, these results suggest that BAIBA has favourable effects on plasma lipids in ob/ob mice with an effect that was more pronounced on phospholipids and cholesterol. However, it is noteworthy that ob/ob mice do not present marked hypertriglyceridemia (Lombardo Y.B., Hron W.T., Sobocinski K.A., Menahan L.A., 1983, Horm Metabol. Res. 16 : 37-42). Accordingly, plasma triglycerides were 1.39 mmol/L in ob/ob mice in the present study, whereas much higher levels (1.96 mmol/L) were found in Swiss mice fed with the western diet (see above). Thus, it remains possible that the beneficial effects of BAIBA on plasma lipids are more pronounced when lipid levels are above a certain threshold. In keeping with this notion, BAIBA reduced by 22% plasma triglycerides in mice fed with the western diet (Figure 6). Moreover, we found that the beneficial effect of BAIBA on plasma lipids was less obvious in very young ob/ob mice (6 weeks at the beginning of the investigations, in contrast to 10-12 weeks for the above-described experiment [Figure 7]) that present lower levels of plasma lipids. Indeed, we found that plasma triglycerides were unchanged (1.07 ± 0.06 and 1.07 ± 0.03 mmol/L, respectively in the control [n=8] and treated [n=8] groups), whereas BAIBA only slightly

reduced plasma phospholipids by 4% (from 4.51 ± 0.09 et 4.35 ± 0.11 mmol/L) and total cholesterol by 5% (from 5.24 ± 0.11 to 4.96 ± 0.15 mmol/L).

CLAIMS

1. A method for the treatment and/or the prevention of diseases linked to the accumulation of triglycerides and cholesterol in tissues and blood comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

2. A method of treatment for lowering the blood levels of cholesterol and/or triglycerides comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

3. The method according to claim 2 for treating or preventing hypertension.

4. A method of treatment for lowering liver triglyceride levels comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

5. The method according to claim 4 for treating or preventing hepatic steatosis and related liver diseases.

6. A method for the treatment or prevention of an obese condition, said method comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

7. A method of treatment for the reduction or inhibition of the gain of body fat comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

8. The method according to claims 1 to 7, wherein the β-aminoisobutyric acid derivative is a salt, an ester or amide thereof.

9. The method according to claims 1 to 8, wherein β-aminoisobutyric acid is of configuration L or D or under a form of a mixture of L and D configurations.

10. The method according to claims 1 to 9, wherein the animal is a human.

11. The method according to claims 1 to 9, wherein the animal is an agricultural animal.

12. The method according to claims 1 to 9, wherein the animal is a domestic animal.

13. Use of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof for the preparation of a pharmaceutical composition intended for the treatment and/or prevention of diseases linked to an accumulation of triglycerides and/or cholesterol in tissues and blood.

14. The use according to claim 13 for the preparation of a pharmaceutical composition intended for the treatment and/or prevention of hypertension, angina pectoris, myocardial infarction and/or hyperlipidemia.

15. The use according to claim 13, for the preparation of a pharmaceutical composition intended for the treatment and/or prevention of hepatic steatosis, steatohepatitis and/or diabetes.

16. The use according to claim 13, for the preparation of a pharmaceutical composition intended for the treatment and/or prevention of the syndrome X (i.e. metabolic syndrome).

17. The use according to claims 13 to 16, wherein β-aminoisobutyric acid is as defined in claim 8 or 9.

18. A pharmaceutical composition comprising at least an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

19. The pharmaceutical composition according to claim 18 for the treatment and/or prevention of hypertension, fatty liver and metabolic syndrome.

20. The pharmaceutical composition according to claim 18 for the treatment and/or prevention of an obese condition.

21. The pharmaceutical composition according to claims 18 to 20, wherein β-aminoisobutyric acid is as defined in claim 8 or 9.

22. The pharmaceutical composition according to claims 18 to 21, comprising a pharmaceutically acceptable carrier or excipient.

23. A nutritional composition comprising an amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof effective to reduce or to prevent an increase in the total body fat mass in human or non human animal.

24. The nutritional composition according to claim 23, wherein β-aminoisobutyric acid is as defined in claim 8 or 9.

25. A method for producing reduction of the fat mass in a human or non-human animal in need thereof, comprising administering thereto an effective amount of nutritional composition according to claim 24.

5

ABREGE

INSTITUT NATIONAL DE LA SANTE ET DE LA ~~RECHERCHE~~ MEDICALE (INSERM)

« Method for the treatment of diseases linked to an ~~accumulation of triglycerides~~ and cholesterol »

The present invention concerns a method for the treatment and/or the prevention of diseases linked to the accumulation of triglycerides and cholesterol in tissues and blood comprising at least the step of administering to a human or non human animal in need thereof, an effective amount of β-aminoisobutyric acid, derivative, prodrug, metabolite or complex thereof.

Controls
BAIBA
% of controls
120
100
80
60
40
20
0
2 weeks
6 weeks
*

Figure 1

Figure 2

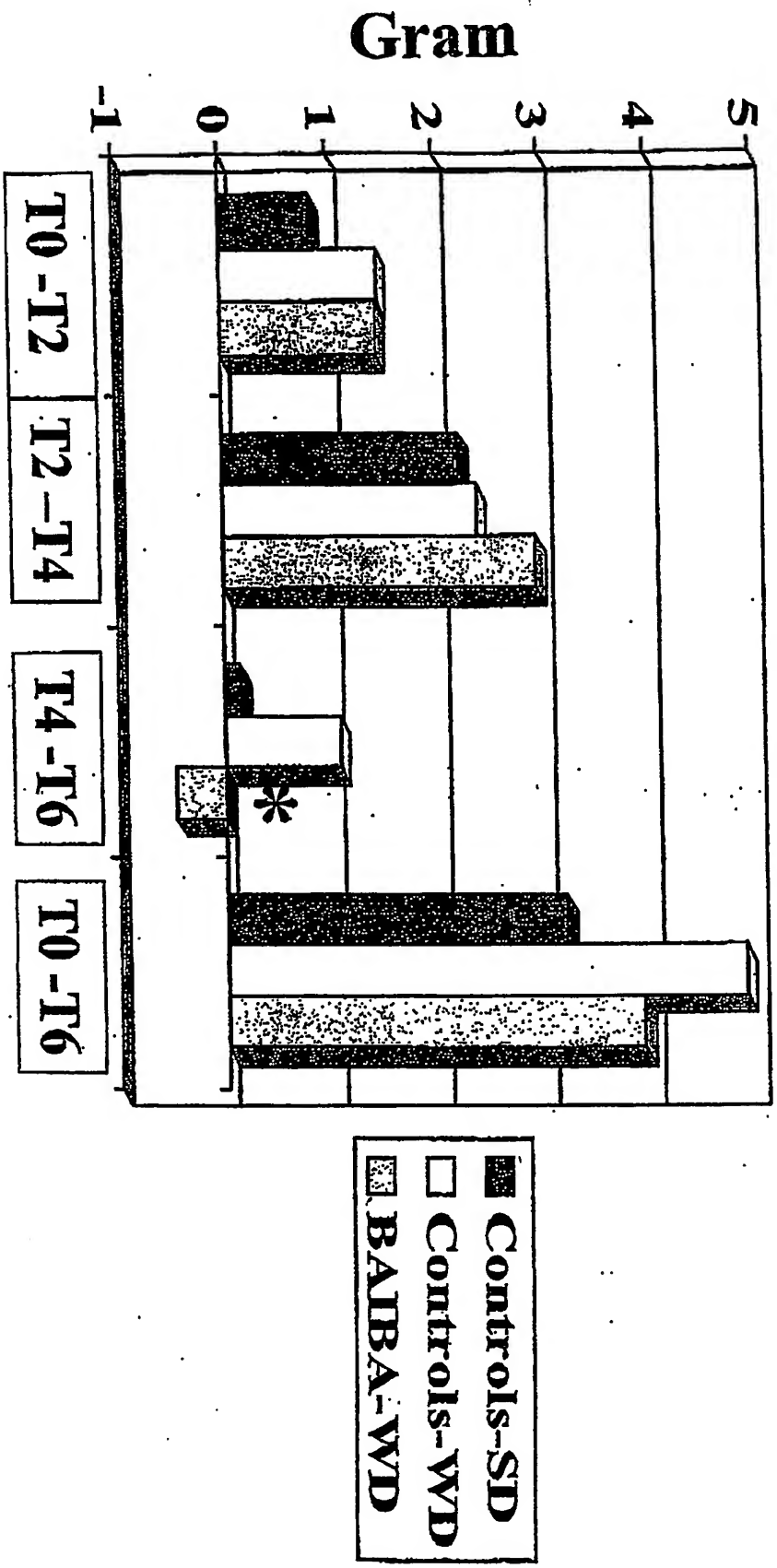
Gram
-1
0
1
2
3
4
5
T0-T2
T2-T4
T4-T6
T0-T6
*
Controls-SD
Controls-WD
BABA-WD

Figure 3

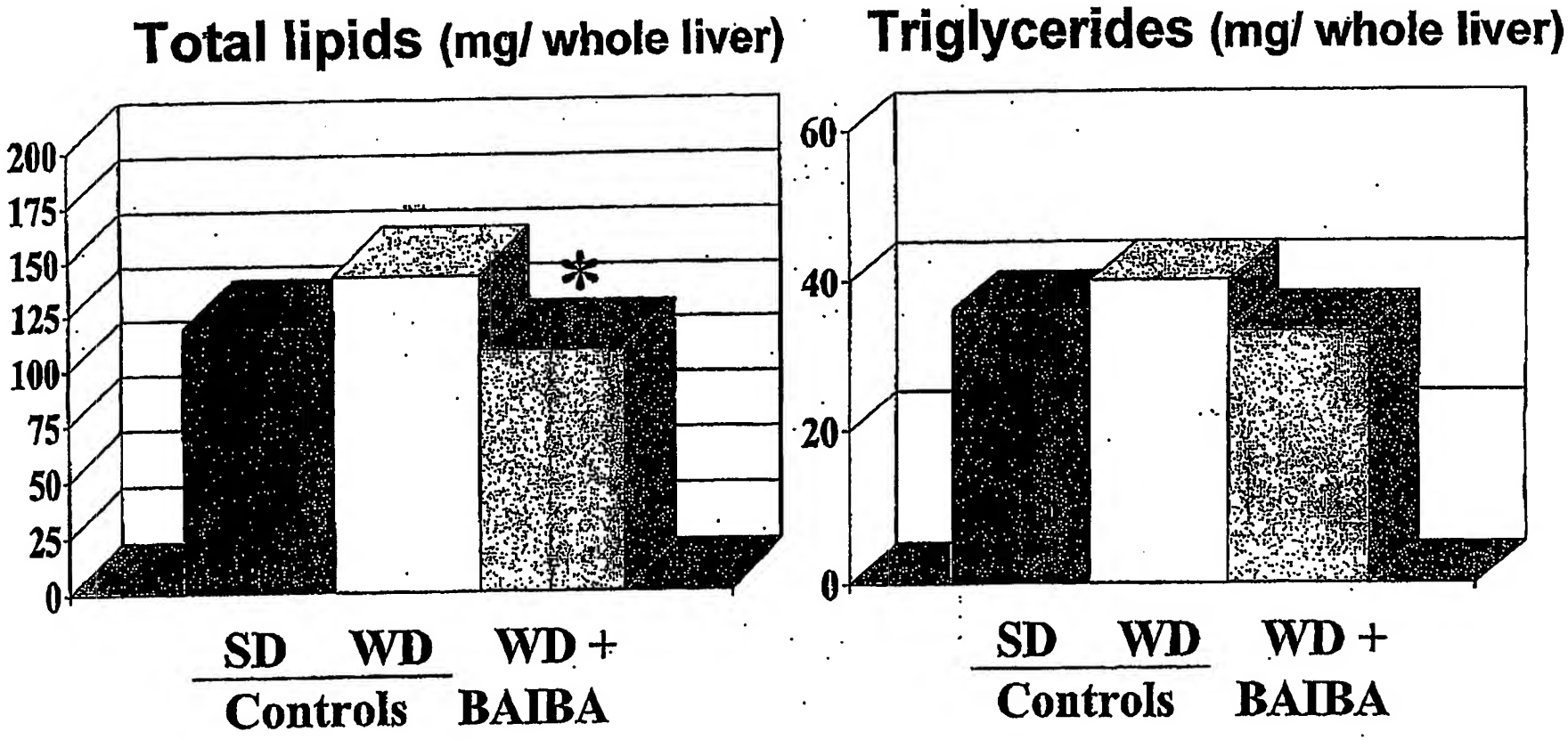
Total lipids (mg/ whole liver)
200
175
150
125
100
75
50
25
0
*
SD
WD
WD +
BAIBA
Controls
Triglycerides (mg/ whole liver)
60
40
20
0
SD
WD
WD +
BAIBA
Controls

Figure 4

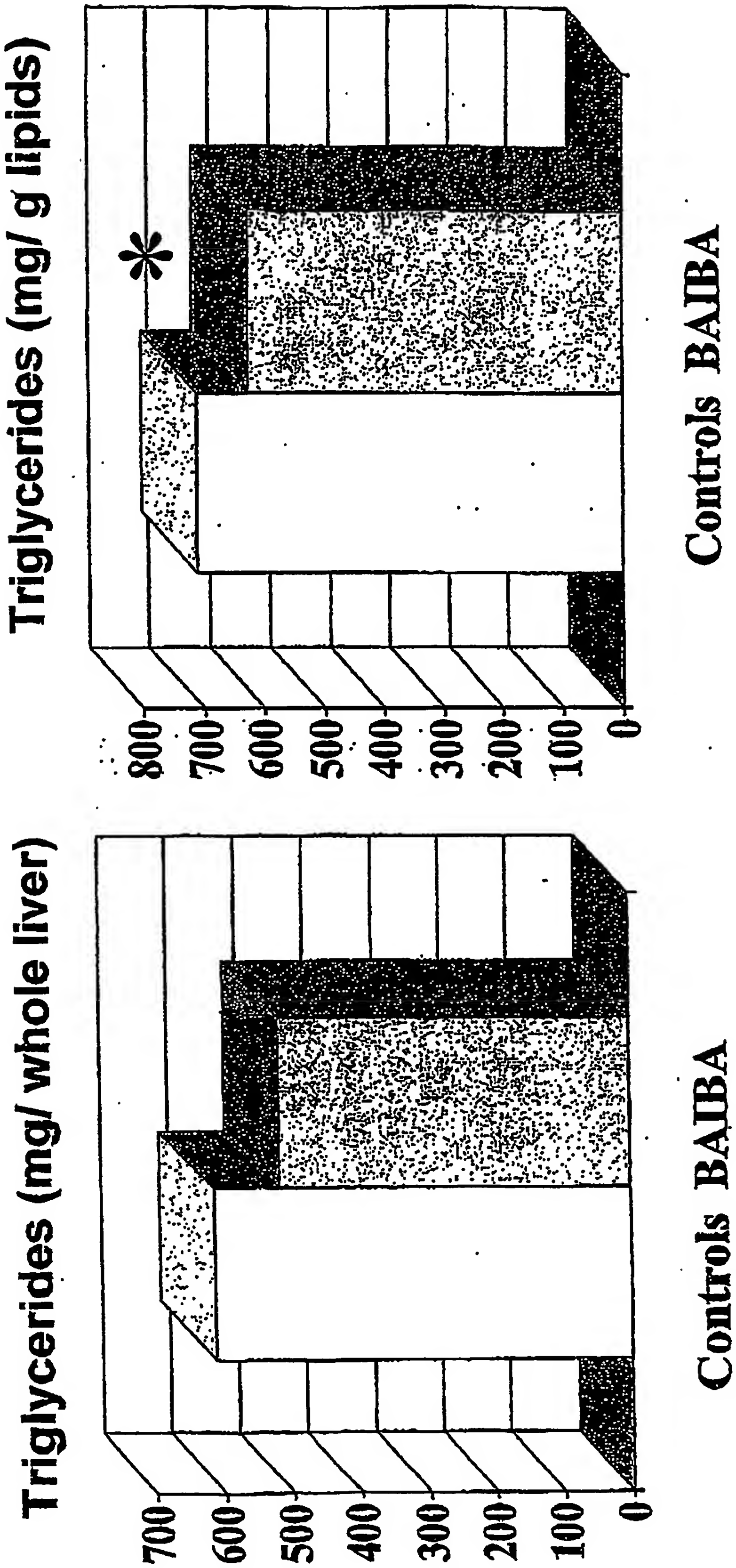
Triglycerides (mg/ g lipids)
*
800
700
600
500
400
300
200
100
0
Controls BAIBA
Triglycerides (mg/ whole liver)
700
600
500
400
300
200
100
0
Controls BAIBA

Figure 5

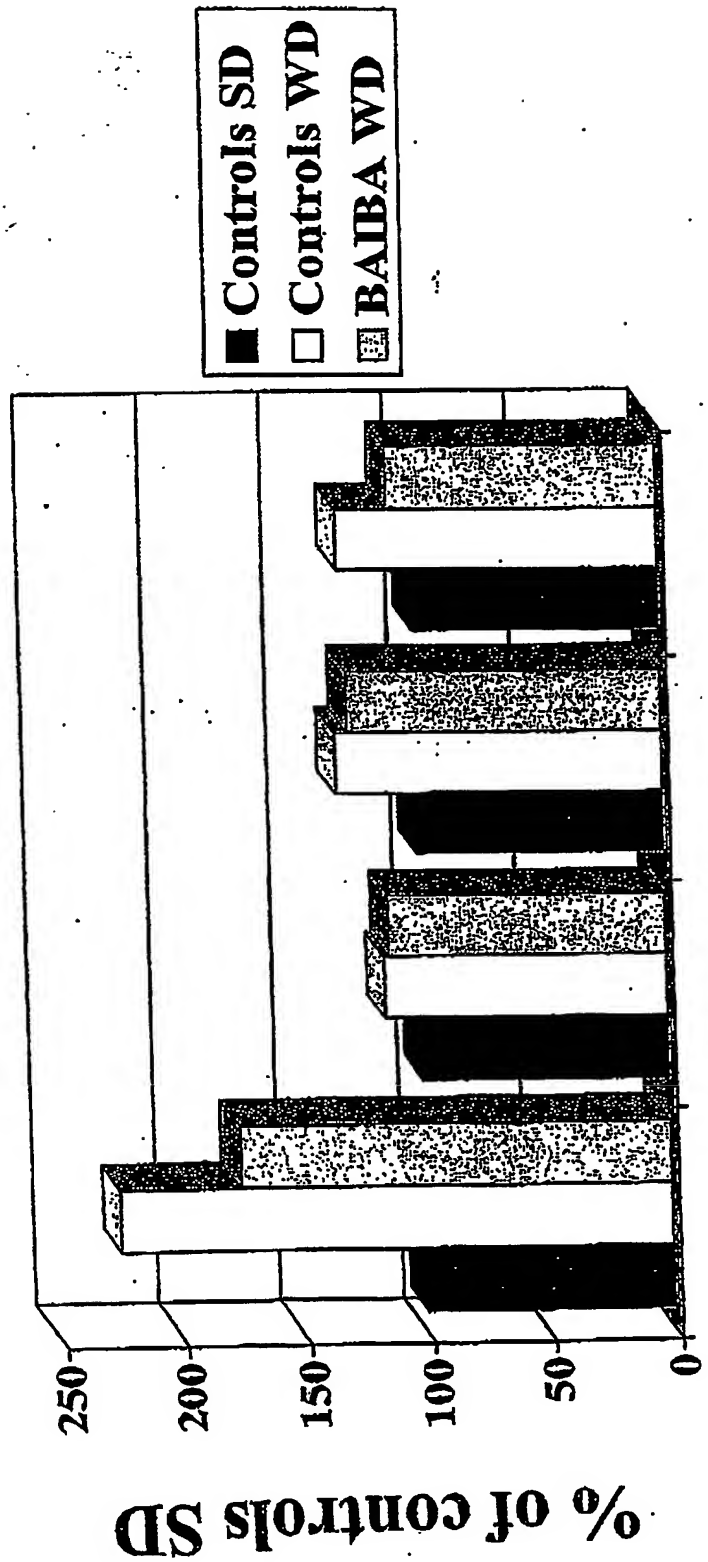
Controls SD
Controls WD
BAIBA WD
% of controls SD
250
200
150
100
50
0
TG
Ph.L
Chol
NEFA

Figure 6

Controls
BAIBA
*
*
100
90
80
70
60
50
40
30
20
10
0
% of controls
TG
Ph.L
Chol

Figure 7